Clinical Pharmacology and Toxicology Review of Tenecteplase, 99-0903

Product: TNK-tPA (TNK, Tenecteplase) (50 mg) with ------ L-arginine, ------phosphoric acid and ---- polysorbate 20. Reconstituted in water for injection. Specific activity of TNK-tPA is 200 units/mg
Indication: Reduction in mortality associated with acute myocardial infarction

Sponsor: Genentech

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Route of administration: intravenous

Clinical Pharmacokinetic Studies

The pharmacokinetics of TNK-tPA were primarily investigated in two studies, N 0647g and N 0660g. These studies are briefly described below:

Study N0647g (TIMI 10A). Open-label, multicenter dose-escalation single IV bolus dose study. Endpoints are pharmacokinetics, pharmacodynamics, safety and tolerance in AMI patients. Dosage is 5, 7.5, 10, 15, 20, 30, 40 and 50 mg.

Study N0660g (TIMI 10B). Randomized, open-label, parallel-group, multicenter, angiographic study of TNK as compared to an accelerated infusion of Activase (15 mg IV bolus, then 50 mg over 30-minute infusion, followed by 35 mg over 60-minutes infusion). Endpoints are pharmacokinetics, safety, and efficacy in AMI patients. Dosages were 30, 40 and 50 mg.

Pharmacokinetics

TNK-tPA has a significantly slower clearance than rtPA (Alteplase). The approximate 4-fold slower clearance permits TNK-tPA to be administered as a bolus rather than an infusion as is the case with rtPA. The pharmacokinetics of TNK are not dose dependent in the range of 30 to 50 mg. In the dose range of 30 to 50 mg clearance of TNK decreased from 216 to 125 ml/min (study N0647g). However, between the range of 5 to 50 mg, the slight decrease in clearance was observed which is not considered to be clinically meaningful. Total body weight correlated with plasma clearance at doses >30 mg. Similarly, an increase in volume of distribution occurred with increased body weight. In the Phase 3 study, dosing normalized by body weight was adopted due, in part, to the observation that patients given a weight adjusted dosage experienced a greater percentage of TIMI grade 3 responses at 90 minutes without an increased risk of bleeding episodes.

In addition to a prolonged rate of exposure for TNK as compared to rtPA, TNK possesses greater fibrin binding and greater resistance to inhibition by plasminogen activator inhibitor type 1 (PAI-1).

Between Phase 1 and Phase 2 clinical studies, a change in the manufacturing technique modified the ------ ---- content of TNK. The difference in ------ acid content resulted in a higher pharmacokinetic exposure to TNK, probably due to a decrease in the percentage of terminal galactose residues.

The results of study N0660g are presented below. This pharmacokinetic study was a randomized, open-label, parallel-group, multicenter, angiographic study of TNK that was compared to an accelerated infusion of Activase (15 mg IV bolus, then 50 mg over 30-minute infusion, followed by 35 mg over 60-minutes infusion). Dosages were 30, 40 and 50 mg.

Dose, mg	N	Cl, ml/mim	Vss, L	T1/2ter, min
TNK 30	48	98± 42	6±3	116±63
TNK 40	31	119±49	8±6	129±87
TNK 50	20	100±32	6±2	90±335
TPA (rh)	53	453±170	29±22	144±100

Table 1. The pharmacokinetics of TNK and rtPA expressed as mean and standard deviation of patient with AMI in Study N0660g. TNK administered as IV bolus and rtPA as an accelerated infusion.

In study N0647g, the dependence of clearance and volume of distribution on dose, total body weight, lean body weight, age and sex was evaluated. A 10 mg increase in dose was found to result in a 17 ml/min decrease in clearance. In study N0660g, a stepwise linear regression of the dosages 30, 40, and 50 mg found that total body weight could account for 19% of the variability in plasma clearance and age accounted for 11% of the variability. A 10 kg increase in body weight resulted in a 9.6 ml/min decrease in plasma clearance.

Pharmacodynamics, clinical

The relationship of TNK to TIMI grade flow at doses of 30, 40, and 50 mg were examined. AUC from 2 to 90 minutes was used as an index of exposure and compared to the percentage of TIMI grade 3. The analysis showed a trend toward increased percentage of subjects obtaining TIMI grade 3 at 90 minutes with increasing exposure. Approximately 40% of the patients demonstrated TIMI grade 3 when AUC was between 220 and 320 ug/ml-min. Seventy-five per cent of the patients obtained a TIMI grade 3 when AUC was between 320 and 430 ug/ml-min. In patients with AUCs >430 ug/ml-min, TIMI grade 3 was observed in 88% of the patents.

Pharmacokinetics, nonclinical

Thirty-two separate studies were submitted regarding the nonclinical pharmacokinetics of various versions of tPA including TNK. The series of studies includes those conducted in various species such as rats, mice, rabbits, dogs and monkeys and considers a number of issues as pharmacological screening, characterization of changes in manufacture, effects of glycosylation and autoradiography. A summary of salient findings is presented below.

Animal studies have demonstrated that liver uptake plays a major role in the clearance of TNK as well as rtPA. Hepatic clearance for some thrombolytics like rtPA primarily operates through high mannose receptors. As TNK was specifically designed to have a reduced high mannose content relative to rtPA, its reduced hepatic clearance is consistent. Nevertheless, both rtPA and TNK are also cleared by other oligosaccharide-independent mechanisms, including low-density lipoprotein receptor proteins.

A number of pharmacokinetic studies were conducted to understanding the relationship between glycosylation and pharmacokinetic behavior in various laboratory animals. These studies, listed in appendix 1, demonstrated the impact of changes in terminal glycosylation on pharmacokinetics.

Pharmacodynamics, nonclinical

Nine studies were submitted on the nonclinical pharmacology of TNK using a variety of animal models as well as normal animals. Among the animal models that were used to establish potential efficacy were the

rabbit model of embolic stroke, electrolytic canine model, and arterial-venous shunt model in the rabbit. Studies were utilized to establish the pharmacodynamic activity of TNK by itself and in comparison to t-PA.

TNK and rtPA have similar activity in models of clot lysis. In the rabbit arterio-venous shunt model, TNK is 3 to 7 fold more potent as compared to rt-PA.

Toxicology

Toxicity studies were conducted in 3 animal species (rat, rabbit and dog). After intravenous administration of up to 50 mg/kg in rats and 30 mg/kg in rabbits and dogs, no unexpected, adverse effects were found.

The following general types of toxicity studies were conducted to establish the safety of TNK

1. Acute toxicity studies in rats, rabbits and dogs.
2. Multidose studies in rabbits, rats and dogs.
3. Special toxicity studies: in vitro hemolysis and blood compatibility, safety pharmacology studies, reproductive toxicology.

Acute toxicity studies were conducted in rats, rabbits and dogs. TNK produced no unexpected findings in rats, rabbits and dogs after IV doses of to 50 mg/kg. Changes in blood coagulation parameters were observed in dogs and rabbits. These changes were decreases in α-antiplasmin and fibrinogen levels and increases in prothrombin time and bleeding from the sties of venipuncture. Antigenicity was observed in rabbits and dogs.

Acute rat study, Study No. 94-0860-0218. A dose of 50 mg/kg of TNK was administered intravenously to SD rats and followed in a 2-week observation period. No evidence of toxicity was observed on body weight, food consumption, or macroscopic findings. Discoloration of the tail due to the direct, thrombolytic effects of TNK at the site of injection was observed.

Acute rabbit study, Study No. 94-092-0218. A dose of TNK was administered intravenously to New Zealand White rabbits and followed in a 2-week observation period. Male and female rabbits were assigned at random to 4 groups (5/sex/group). Each group was administered vehicle, 0.3, 3.0 or 30 mg/kg of TNK. No evidence of toxicity was observed on body weight, food consumption, or macroscopic findings. TNK produced a dose dependent increase prothrombin time and decrease in fibrinogen and alpha 2-antiplasmin. By day 14, rabbits developed antibodies to TNK in a roughly dose proportionate manner. Mean anti-TNK titers were 2 for 0.3 mg/kg, 3 for 3 mg/kg and 4 for 30 mg/kg.

Acute dog study, Study No. 94-090-0218. TNK was injected into Beagle dogs as a single IV bolus on day 1 and followed by a challenge dose on day 14 to some groups of animals. Animals were observed for a total of 21 days. Thirty-two dogs were randomized to 8 groups of 2 animals/sex/group. Animals in groups 1 though 4 received a challenge on dose on day 14. Dosage for TNK by groups were as follows - vehicle, 0.3, 3, 30 mg/kg,; vehicle + aspirin + heparin, 0.3 mg/kg + aspirin + heparin; 3 mg/kg + aspirin + heparin; 30 mg/kg + aspirin + heparin. No unexpected evidence of toxicity was observed on body weight, food consumption, or macroscopic findings. At doses of 30 mg/kg, bleeding from the mouth and puncture sites were observed. A dose dependent prolongation occurred in prothrombin times and activated partial thromboplastin time; additionally a dose dependent decrease in fibrinogen and alpha 2-antiplasmin was measured. Angioedema was observed in the dogs due to the presence of arginine in the vehicle. A single dose of TNK was studied .in combination with either acetyl salicyclic acid (162.5 mg, given orally 24 and 2 hours prior to TNK) or unfractionated heparin given at 100 units/kg iv bolus and followed by an approximate 4-hour infusion at 1 ml/hr of 50 units/kg/hr. No interactive effects were observed on TNK in terms of blood coagulation endpoints, additional toxicity or pharmacokinetics. An antigenic response was observed to TNK. Titers of antibodies to TNK were approximately dose dependent. Titers of >2 were observed in dogs given 3 mg/kg or higher by day 14. By day 18, all groups showed an antigen response, which ranged from 1.7 to 4.4. Following administration on day 14, group 4 (30 mg/kg) males died within 45 minutes of dosing. Because of the death in the males, female dogs were not dosed on day 14. Lower dosage groups also

exhibited evidence of immune-based responses to TNK. The pharmacokinetics of TNK were measured as a terminal half-life computed from the last 3 blood samples taken during the study and fitted to a log-linear regression. Half-lives across the dose groups were for 0.3 mg/kg – 25 minutes, for 3 mg/kg – 32 minutes and

Acute Intravenous Tolerance Study with GN0218 in Rabbits, Study No. 94-089-0218. A study in New Zealand White rabbits was conducted to assess the local tolerance of IV bolus administration. A single 1 ml dose of 5 mg/ml or vehicle was injected in the marginal ear vein to 9 animals per group. Three groups of animals were sacrificed and necropsied on days 2, 4, and 8. Local redness and swelling, accompanied subcutaneous hemorrhage at the site of injection. Areas of irritation were observed in one control and eight of nine treated animals. Microscopically, minimal hemorrhage was seen in one of nine control animals and minimal to slight hemorrhage in four of nine treated animals. Slight subacute inflammation occurred at a perivascular site in one treated and one control animals. No evidence of irritation to the venous endothelium was seen. The most severe effects occurred on days 1 and 2 of the study. Recovery had occurred by day 8 of the study.

Local Intra-arterial Tolerance of Tenecteplase in Rabbits, Study 98-304-0218. The local tolerance of a single, intra-arterial bolus injection of 0.5 ml was investigated in New Zealand rabbits. Hematomas were observed at the site of injection.

Local Paravenous Tolerance of Tenecteplase in Rats, Study No. 98-305-0218. The local tolerance after a single, paravenous bolus injection of 0.2 ml was investigated in Chbb:THOM rats. TNK (5 mg/ml) or placebo was administered paravenoulsy medially and laterally in relation to the jugular vein (group size N=4 sex/group). Slight reddening was found in one female animal and slight hemorrhaging in the paravenous area was noted in 3 animals given TNK.

Multidose toxicity studies were conducted in rats, rabbits and dogs.

Multidose rat study, No. 94-087-0218. TNK was administered subcutaneously, daily at 0 (vehicle), 0.3, 1, 3, or 10 mg/kg to SD rats for 15 days. The vehicle control and highest dose groups were composed of 15 animals/sex per group; other groups were composed of 10 animals/sex per group. Five animals in the control and highest dosed groups were designated as recovery groups and followed for 2 weeks after the cessation of dosing. No effects were observed on clinical signs of toxicity, body weight, food consumption, ophthalmic findings, organ weights, or macroscopic or microscopic pathology. At 10 mg/kg TNK was associated with higher serum levels of protein, albumin, and calcium; higher levels of cholesterol for males and lower alkaline phosphate levels for female animals. Discoloration was observed at the site of injection. By day 16 an antigenic response was observed which was dose related. Animals given 0.3, 1.0 or 3.0 mg/kg developed low titers to TNK with an incidence of 10% to 25% per group dose; whereas rats given 10 mg/kg had an incidence of 40%. A recovery group of animals given 10 mg/kg experienced an incidence of 78% by day 29/30 of the study.

Multidose dog study, No 94-091-0218. TNK was administered intravenously, daily at 0.3 mg/kg to Beagle dogs for at least 9 days. Daily iv bolus administration occurred at 1, 3, or 10 mg/kg for at least 8 days or daily 90-minute infusion of 10 mg/kg for 14 days. Mean cumulative body weight gain from day 1 to day 8 was slightly reduced in a dose dependent manner. No effects were observed on EKG, heart rate, blood pressure or ophthalmic endpoints. No pharmacokinetic accumulation was observed. At 10 mg/kg the following effects were observed: prolongation in bleeding times, bleeding from venipuncture sties and changes in indices of clotting. Angioedema was observed as swelling of the face, ears, limbs. Additionally, pruritis, red skin, and hive-like reactions were present that were indicative of an immune response to TNK. Antibodies were detected in the treated dogs. Daily administration at doses of 1 mg/kg or higher produced hemorrhage in the liver, gall bladder, stomach, diaphragm and lymph nodes.

Pilot 2-Week Intravenous Toxicity Study with GN0210 (Activase) in Beagle Dogs, Study No. 93-539-0210. To provide comparative data for TNK, Activase was administered to dogs (2 dogs/sex/group) in a repeat

dose study. Activase was injected as daily 90-infusions of 30 mg/kg for at least 7 days, or 3 and 10 mg/kg for 14 days. No effects were seen on body weight, EKG, or body temperature. Dogs given the Activase vehicle or Activase exhibited signs of hypoactivity, vomiting, swollen faces or limbs, tremors, lameness, red skin, and nonformed or mucoid feces. Swelling of the face and limbs were related to arginine. Changes in blood coagulation parameters occurred in a dose-dependent manner. At 10 and 30 mg/kg Activase produced prolonged bleeding times and changes in clinical pathology associated with decreased clotting (increased prothrombin time, activated platelet partial thromboplastin time and fibrin/fibrinogen degradation products with decreased fibrinogen. Animals given 30 mg/kg experienced mild decreases in red blood cell count, hemoglobin, and hematocrit. Dosing was discontinued prior to completing all 30 mg/kg dosing due to severe hypotension by day 9, which coincided with the development of antibodies.

Antibody Response of Daily Administered Tenecteplase in Female Rabbits, Study No. 96-361-0366. The time course of antibody development to TNK was followed after IV administration in female rabbits. Animals were given 1, 3, or 10 mg/kg for 13 days. Daily administration of TNK produced an antibody response in all treated animals by day 8.

Special Toxicity Studies

In Vitro Hemolytic Potential and Blood Compatibility Testing with GN0218 (Tenecteplase), Study No. 94-088-0218. When equal amounts of whole blood, serum or plasma were mixed with 5 mg/ml of TNK or vehicle, no evidence of hemolysis was observed in RBC's from humans or dogs.

Safety Pharmacology Studies

Cardiovascular and Respiratory Safety Pharmacology with Tenecteplase in Anesthetized Rabbits, Study No. 98-302-0218. A cardiovascular and respiratory safety pharmacology study was conducted in anesthetized New Zealand White rabbits. Using doses of 0.03 to 3 mg/kg, no effects were found on systolic arterial blood pressure, heart rate, left ventricular pressure and dP/dt max, respiratory rate, tidal volume, inspiratory peak flow and expiratory peak flow over 30 minutes after iv injection.

Cardiovascular Safety Pharmacology with Tenecteplase in Conscious Cynomolgus Monkeys, Study No. 97-066-0218. A cardiovascular safety pharmacology study was conducted in free-moving, monkeys. Doses were administered in an intra-subject dose escalation scheme. No effects were seen after doses of 0.003 to 0.3 mg/kg. At a dose of 3 mg/kg an immediate rise in arterial blood pressure from 10% to 22% was observed between 5 and 15 minutes after injection which persisted for approximately 30 minutes. No changes were seen in EKG waveforms. After 30 mg/kg a decrease in arterial blood pressure was observed that was concomitant with an increase in heart rate (approximately 17% to 32%). EKG revealed an inversion in 1 animal or attenuation in 2 animals. The dose of 30 mg/kg resulted in ataxia in all animals or cardiovascular collapse. Upon postmorten examination, animals were found to have evidence of pulmonary edema and congestion possibly due to localized hemorrhage. The 30 mg/kg dose is approximately 60 times the intended clinical dose
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Renal Safety Pharmacology with Tenecteplase in Dogs, Study No. 98-306-0218. A renal safety pharmacology study was conducted in Beagle dogs using doses up to 9 mg/kg. After IV injection of saline, TNK, TNK vehicle, the following parameters were measured: urine volume, pH and chemistry (sodium ion, potassium ion, chloride ion), creatinine and glucose. The following changes were found: slight increase in urine volume, retention of sodium, decrease in chloride excretion and increase in potassium excretion. Doses of 1 or 3 mg/kg did not significantly change electrolyte excretion. These findings occurred in dogs that undergo an angioedemic response to the arginine in TNK and were observed in the dogs undergoing study.

Behavioral Safety Pharmacology with Tenecteplase in Mice, Study No- 98-303-0218. After the injection of doses of up to 10 mg/kg, to mice a behavioral safety pharmacology study was conducted using the method of Irwin. After a single IV dose, body temperature, exploratory motility and pain perception were assessed. A dose-dependent increase in the loss of landing reflex and/or loss of grasping reflex was observed up to 24

hours after dosing, but no evidence of muscle relaxation were found. Exploratory motility was increased at 1 and 3 mg/kg, but not at 10 mg/kg. Rectal body temperature was slightly increased following 3 mg/kg without evidence of a dose response relationship.

Developmental Toxicity Studies

Due to the development of antibodies to TNK, dosing during the developmental toxicology studies were fractionated into consecutive phases.

Developmental Toxicity Study in Rabbits, Study No. 96-440-0218. A rabbit developmental toxicity study using consecutive dosing periods to cover the period of organogenesis (gestation days [GD] 6 to 18) was conducted with three, 5day periods. Pregnant New Zealand White (NZW) rabbits were assigned to 10 groups (N=18 animals/group). Group 1 was given TNK vehicle on GDs 6 to 18. Groups 2, 3 and 4 were given 0.5, 1.5 or 5 mg/kg of TNK on GDs 6 to 10. Groups 5, 6, 7 were given 0.5, 1.5 or 5 mg/kg of TNK on GDs 11 to 14. Groups 8, 9, and 10 were given 0.5, 1.5 or 5 mg/kg of TNK on GDs 15 to 18. In group 1, a total of 16 of 18 rabbits died on GDs 16 to 20 and 1 rabbit aborted on GD 17. Deaths appeared associated with vaginal hemorrhage. In groups 2, 3, and 4, doses of up to 5 mg/kg did not produce maternal or developmental toxicity. In groups 5, 6, and 7, maternal death was observed in 17 of 18, 16 of 18 and 17 of 18 rabbits; most deaths occurred on GD 14. Deaths were associated with hemorrhage. In groups 8, 9, and 10, 100% mortality occurred. Subsequent to the studies cited above additional groups to put on study to investigate the death of dams in the vehicle control group. TNK vehicle or saline was injected in groups 11 (saline, GD 6 to 18), 12 (TNK GD 6 to 10), 13 (TNK GD 11 to 14), and 14 (TNK GD 15 to 18). In these additionally groups, TNK vehicle was not found to cause maternal or developmental toxicity and suggest the initial evidence of TNK vehicle toxicity was due to mis-dosing with TNK itself during the study. To further study the possible effects of the TNK vehicle on developmental toxicity, 2 more studies were conducted using L or D-arginine (Study Nos. 97-177-0218 and 97-234-0218. L-Arginine is a component of the TNK vehicle. In these studies NZW rabbits were dosed on GD 6 to 18 without evidence of maternal or developmental toxicities.

Multiple vs. Single Tenecteplase Administration to Pregnant Rabbits, Study No 97-244-0218 (This study is titled, “The effect single and multiple administration of TNK-tPA on pregnancy in rabbits on gestation days.13 – 17”). To determine whether single or multiple dosing of TNK would produce maternal or developmental mortality, pregnant NZW rabbits were dosed using 5 different groups (N=4/group). Group 1 was given TNK at 5 mg/kg from GD 13 to 17. Groups 2, 3, 4, and 5 were given single doses of TNK at 5 mg/kg on GD 14 to 17 (group 2 on day 14, group 3 on day 15, group 4 on day 16 and group 5 on day 17). No adverse effects occurred in rabbits given a single injection whereas both perivaginal bleeding and death occurred in Group 1 rabbits.

The effects of various TNK-tPA vehicle formulations on the pregnancy of rabbits. Study No. 97-177-0218. Pregnant New Zealand White rabbits were given intravenous doses of TNK-tPA vehicle with L-arginine, D-arginine or saline at 1 ml/mg on gestation days 6 to 18. Each 1 ml of vehicle was composed of isotonic saline, 300 mM L-arginine H3PO4, pH 7.3, 0.025% Tween 20 or 300 mM D-arginine H3PO4, pH 7.3, 0.025% Tween 20. No evidence of toxicity was observed.

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